Antidepressant-Like Effect of Venlafaxine Is Abolished in μ-Opioid Receptor–Knockout Mice

Soichiro Ide1,2, Shunsuke Fujiwara2, Masayuki Fujiwara2, Ichiro Sora3, Kazutaka Ikeda4, Masabumi Minami1, George R. Uhl5, and Kumatoshi Ishihara2,*

1Department of Pharmacology, Graduate School of Pharmaceutical Sciences, Hokkaido University, Sapporo 060-0812, Japan
2Laboratory of Neuropharmacology, Faculty of Pharmaceutical Sciences, Hiroshima International University, Kure 737-0112, Japan
3Department of Biological Psychiatry, Tohoku University Graduate School of Medicine, Sendai 980-8574, Japan
4Division of Psychobiology, Tokyo Institute of Psychiatry, Tokyo 156-8585, Japan
5Molecular Neurobiology, National Institute on Drug Abuse, Baltimore, MD 21224, USA

Received May 17, 2010; Accepted July 12, 2010

Abstract. Although the opioid system is known to modulate depression-like behaviors, its role in the effects of antidepressants is not yet clear. We investigated the role of μ-opioid receptors (MOPs) in the effects of venlafaxine, a serotonin and norepinephrine reuptake inhibitor, in the forced swim test using MOP–knockout (KO) mice. Venlafaxine reduced immobility time in wild-type mice (C57BL/6J), but not in MOP-KO mice, although no significant effects were observed on locomotor activity. These results suggest that MOPs play an important role in the antidepressant-like effects of venlafaxine.

Keywords: μ-opioid receptor, knockout mouse, antidepressant
the Institutional Animal Care and Use Committee, and all animal care and treatment were in accordance with our institutional animal experimentation guidelines. Naive adult (>10-week-old) male mice were group-housed in an animal facility maintained at 22 ± 2°C and 55 ± 5% relative humidity under a 12/12-h light/dark cycle with lights on at 8:00 am and off at 8:00 pm. Food and water were available ad libitum. All behavioral tests were conducted between 1:00 pm and 6:00 pm.

Venlafaxine hydrochloride (LKT Laboratories, St. Paul, MN, USA) was dissolved in saline and injected in volumes of 10 ml/kg.

For the forced swim test, animals were forced to swim in a cylindrical Plexiglas tank (30-cm height × 30-cm diameter) containing 20-cm-deep water for 6 min per day for 5 consecutive days. The water temperature was maintained at approximately 25°C. Immobility time was recorded with an animal activity monitoring apparatus equipped with an infrared detector (SUPERMEX, CompACT FSS; Muromachi Kikai Co., Tokyo). After each session, the mice were immediately removed from the cylinder, dried with a towel, and kept under a heating lamp until completely dry, before being returned to their home cages. Venlafaxine and saline were administered subcutaneously 20 min before each daily test.

Locomotor activity was assessed with an animal activity monitoring apparatus equipped with an infrared detector (SUPERMEX, CompACT FSS, Muromachi Kikai Co.). Mice were placed individually in 30 × 45 × 30 cm plastic cages, to which they had not been previously exposed, under dim light and sound-attenuated conditions. Locomotor activity was monitored for 30 min. Venlafaxine and saline were administered subcutaneously 20 min before the test.

Data were analyzed with analysis of variance (ANOVA) followed by the Student-Newman-Keuls post hoc test. Values of $P < 0.05$ were considered statistically significant.

To evaluate the antidepressant-like effects of venlafaxine, immobility time during the 6 min, 5-consecutive-day forced swim test was analyzed in wild-type and MOP-KO mice (Fig. 1). Two-way, repeated-measures ANOVA of total immobility time during the 6-min test on each of the 5 days with drug dose revealed that immobility time significantly decreased after venlafaxine treatment in wild-type mice ($F_{2,100} = 13.2, P < 0.001$), but not in MOP-KO mice (Fig. 1: A and B). Post hoc comparisons revealed that venlafaxine treatment (10 mg/kg) reduced immobility time in wild-type mice ($P < 0.05$) from Day 1 to 4. Venlafaxine treatment (30 mg/kg) also reduced immobility time in wild-type mice ($P < 0.05$) on Day 3 and 4. In the saline-treated group, two-way, repeated-measures ANOVA of immobility time with genotypes of mice revealed that immobility time was significantly shorter in MOP-KO mice than wild-type mice ($F_{1,18} = 9.4, P < 0.01$), similar to our previous report (9). Immobility time in MOP-KO mice was significantly shorter than that in wild-type mice on Day 2 ($F_{1,18} = 8.2, P < 0.05$) and Day 3 ($F_{1,18} = 14.7, P < 0.005$). Two-way, repeated-measures ANOVA of immobility time with genotypes of mice also revealed no significant differences between genotypes in either the 10 mg/kg venlafaxine- or 30 mg/kg venlafaxine–treated groups.

To test the possible influences of motor dysfunction on the antidepressant-like effects of venlafaxine, locomotor activity in both wild-type and MOP-KO mice was analyzed (Fig. 2). Venlafaxine showed no significant effects on locomotor activity in either wild-type or MOP-KO mice.

![Fig. 1. Effect of venlafaxine on immobility time in the forced swim test in wild-type and MOP-KO mice. Animals were subjected to daily 6-min tests for 5 consecutive days. The figure shows the cumulative immobility time during the 6-min tests over 5 days in wild-type mice that received saline (n = 12) or venlafaxine (10 mg/kg, n = 9; 30 mg/kg, n = 7) (A) and MOP-KO mice that received saline (n = 8) or venlafaxine (10 mg/kg, n = 6; 30 mg/kg, n = 6) (B). *P < 0.05, significant difference from corresponding value in the saline-treated group. Data are expressed as the mean ± S.E.M.](image-url)
Effects of Venlafaxine in MOP-KO Mice

In the present study, venlafaxine reduced immobility time in wild-type mice in the forced swim test, an effect that was abolished in MOP-KO mice. These results suggest that MOPs play an important role in the antidepressant-like effects of venlafaxine. This is consistent with previous reports showing that the antidepressant-like effects of venlafaxine in the forced swim test in mice were antagonized by naloxone, a nonselective opioid antagonist (10), although selective antagonists for each opioid subtype were ineffective. Venlafaxine is a nontricyclic antidepressant that inhibits both 5-HT and norepinephrine reuptake and has no binding affinity for opioid receptors (11). Venlafaxine blocks 5-HT uptake at low doses and norepinephrine uptake at higher doses, and the doses of venlafaxine used in the present study (10 and 30 mg/kg) may act on both 5-HT and norepinephrine transporters (12). Thus, the indirect modulation of 5-HT and norepinephrine neurotransmission by endogenous opioid neurotransmission via MOPs may be hypothesized to be involved in the antidepressant-like effects of venlafaxine.

The locus coeruleus is hypothesized to be one of the most important brain regions implicated in stress, depression, and the mechanisms of action of antidepressants. Stress and depression activate the hypothalamic–pituitary–adrenal axis and increase norepinephrine release in the locus coeruleus. Norepinephrine release in the locus coeruleus is partially regulated by both opioid and noradrenergic mechanisms (13). The locus coeruleus also receives dense 5-HT projections from the dorsal raphe and pericoerulear region (14). Moreover, acute administration of venlafaxine exerted an inhibitory effect on the spontaneous activity of locus coeruleus neurons (15). Thus, a possible mechanism of action of the antidepressant-like effects of venlafaxine may be inhibition of locus coeruleus activity, which may be regulated by endogenous norepinephrine, 5-HT, and opioid systems. Deletion of MOPs might suppress the molecular control of locus coeruleus neurotransmission and depression-like responses in MOP-KO mice.

In the present study, MOP-KO mice showed reduced immobility in the forced swim test, which is consistent with our previous report showing decreased immobility in both the tail-suspension and forced swim tests and reduced stress-induced plasma corticosterone concentrations in MOP-KO mice compared with wild-type mice (9). Thus, MOP-KO mice may be resistant to the stress stimulus and be in an antidepressive-like state. Further studies may reveal the changes in neurotransmission that suppress depression-like responses and abolish the antidepressant-like effect of venlafaxine in MOP-KO mice.

In conclusion, the antidepressant-like effects of venlafaxine were abolished in MOP-KO mice, with no effect on locomotor activity. These results suggest that MOPs play an important role in the modulation of the antidepressant-like effect of venlafaxine.

Acknowledgments

We thank M. Arends for editing the language of the manuscript. This study was supported by the Naito Foundation, the Suzuken Memorial Foundation, and the U.S. National Institute on Drug Abuse Intramural Research Program.

References


